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Application News

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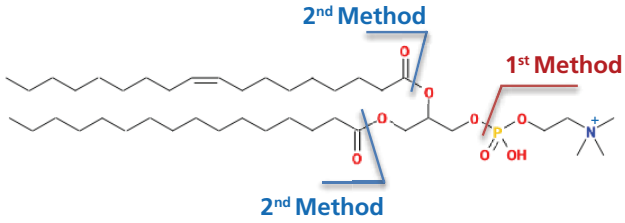
Liquid Chromatography Mass Spectrometry

Development of a Phospholipid Profiling Method Using Triple Quadrupole LC/MS/MS

The lipid bilayer membrane structure of cell membranes is formed of phospholipids, with fatty acid chains oriented inside the membrane and polar groups situated on the membrane surface. Precursors of physiologically active lipids bound to phospholipids as fatty acids include polyunsaturated fatty acids such as arachidonic acid, EPA, and DHA. These lipids contribute to the formation of a wide variety of membrane structures. Due to recent reports of a causal association between phospholipid compositions and various diseases, phospholipid profiling techniques have gained interest as an important approach in disease marker screening and disease mechanism identification.

Shimadzu has created a phospholipid profiling LC/MS/MS MRM library for the classification of phospholipids in biological samples. Phospholipids are divided into glycerophospholipids and sphingophospholipids. Qualitative analysis of phospholipids by MS/MS involves phospholipid classification via the detection of product ions created by polar group elimination, such as choline and ethanolamine elimination, and subsequent phospholipid structure prediction based on molecular ions (Fig. 1). The Shimadzu MRM library includes 422 constituents (1st method), and the phospholipid targets of the library are phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidylglycerol (PG), phosphatidylinositol (PI), and sphingomyelins (SM). A list of the fatty acids included as analytical targets are shown in the table on the right in Fig. 1. A single chromatographic analysis can be used to profile 422 phospholipid constituents. The MRM library also includes 867 MRM transitions that are needed to determine the fatty acid composition. The phospholipid profiling workflow begins with the 1st method, after which the 2nd method is performed if the fatty acid composition analysis is needed. Shimadzu has also created the MRM Event Link Editor that edits MRM methods, and is needed to create a 2nd method from the 867 MRM library transitions.

The library allows for easy phospholipid profiling with a triple quadrupole mass spectrometer, and stress-free fatty acid composition analysis.



	Number of Double Bonds					
Carbon Number	C14:0	C14:1				
	C16:0	C16:1				
	C18:0	C18:1	C18:2	C18:3		
	C20:0	C20:1	C20:2	C20:3	C20:4	C20:5
	C22:0	C22:1	C22:6			

Fig. 1 PS (16:0/18:1) structure and fragmentation points (left), and tabulated list of fatty acids (right). Here, the 1st method refers to MRM that analyzes product ions obtained by polar head group elimination, and the 2nd method refers to MRM that analyzes fatty acid product ions.

Fig. 2 Workflow of MRM based phospholipid profiling. Structural information obtained from the 1st method comprises the polar head group, and the total carbon number of the fatty acids and number of double bonds (eg: PC (34:1)). The 2nd method is used to determine the fatty acid composition of the constituent obtained in the 1st method, such as PC (16:0/18:1) from PC (34:1).

HPLC Conditions

Analytical Column	: Phenomenex Kinetex C8 (150 mm L × 2.1 mm I.D., 2.6 µm)
Mobile Phase A	: 20 mM ammonium formate
Mobile Phase B	: Acetonitrile/Isopropanol (1:1)
Time Program (B %)	: 20 % (0 min) → 20 % (1 min) → 40 % (2 min) → 92.5 % (25 min) → 100 % (26 min) → 100 % (35 min)
Flowrate	: 0.3 mL/min
Injection Volume	: 3 µL
Column Oven Temperature	: 45 ˚C

MS Conditions (LCMS-8050)

Ionization Method	: ESI (Positive/Negative)
Nebulizer Gas Flowrate	: 3.0 L/min
Drying Gas Flowrate	: 10.0 L/min
Heating Gas	: 10.0 L/min
DL Temperature	: 250 ˚C
Heater Block Temperature	: 400 ˚C
Interface Temperature	: 300 ˚C
CID Gas Pressure	: 230 kPa

The LCMS-8050 system was used with a phospholipid MRM library 1st method (MRM of 422 phospholipid constituents) to profile a lipid extract obtained from mouse brain. As a result, the peaks were detected for 130 constituents, and the peak heights of 102 constituents were 10,000 or above.

MRM Event Link Editor was used to create a 2nd method, which is needed to determine the fatty acid composition of the peaks detected by the 1st method, and analysis was performed. Taking PC (38:4) as an example, the MRM Event Link Editor software creates a method from the MRM transitions expected for all possible combinations of fatty acids (18:0/20:4, 18:1/20:3, 18:2/20:2, 18:3/20:1) in ESI negative mode (ESI (-)), and takes those transitions from the 867 MRM transitions in the MRM library. The 2nd method would also include the MRM transitions used in the 1st method to monitor for eliminated polar head groups.

For the 33 possible fatty acid compositions, the peak areas obtained from MRM monitoring of polar head groups are shown in the vertical axis in Fig. 3. For PC (38:4), the MRM chromatogram obtained from the 2nd method is shown on the bottom left of Fig. 3. The MRM chromatogram obtained when monitoring for the polar head group (choline), which is shown above the 2nd method chromatogram, detected a main peak at 15.5 minutes. The MRM chromatogram below is for fatty acid product ions with compositions of 18:0 (stearic acid) and 20:4 (arachidonic acid). The MRM chromatograms obtained for other fatty acid combinations did not detect a peak at 15.5 minutes, showing that the main fatty acid combination is 18:0/20:4.

This article shows the LC/MS/MS MRM library (phospholipids) can be used for easy phospholipid profiling and fatty acid composition determination.

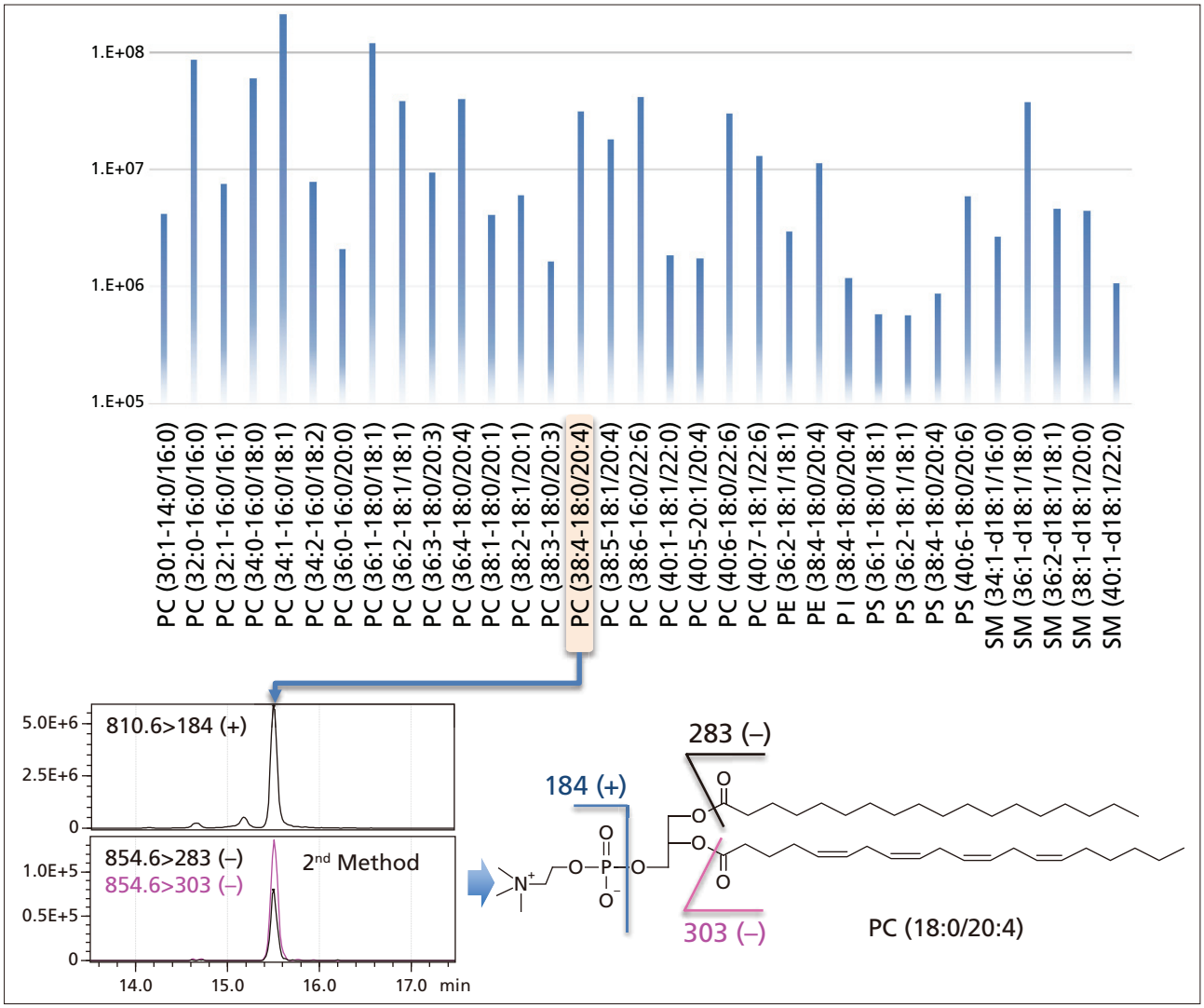


Fig. 3 Profiling results for PC in mouse brain lipid extract (top image). The MRM chromatogram used to determine the fatty acid composition of PC (38:4) (bottom left) showed the fatty acid composition was 18:0/20:4 (bottom right).

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